

## Lab (2)

## Quantitative amino acids estimation by ninhydrin method

 **Materials:****Chemical**


Standard amino acid stock solution (100 µg/ml), 8% w/v of ninhydrin reagent, 50% v/v ethanol, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, aluminum foil, plastic cuvette, spectrophotometer, water bath.

**Protocol:****1. Set up 7 test tubes as following:**

Tube	Standard amino acid solution (100 µg/ml) (ml)	Distilled water (ml)	Unknown sample	Ninhydrin reagent (ml)
<b>Blank</b>	-	4		1
<b>A</b>	1.2	2.8		
<b>B</b>	1.6	2.4		
<b>C</b>	2	2		
<b>D</b>	2.4	1.6		
<b>E</b>	2.8	1.2		
<b>Unknown sample</b>	-	-	4	

- Mix the contents of the tubes by vertexing/shaking the tubes.
- Cover the tubes with aluminium foil.
- Place all the test tubes in 80°C for 15 minutes.
- Cool the test tubes in cold water, then add 1 ml of ethanol to each test tube and mix well.
- Record the absorbance of all tubes against the blank at 570 nm using a colorimeter (spectrophotometer).
-  **PAUSE AND THINK** → What the blank should contain? Why?
- Calculate the amino acid concentration for each standard amino acid solution using  $C_1 \times V_1 = C_2 \times V_2$  formula.
- Plot standard curve for absorbance against amino acids concentration (µg/ml) using results for solutions (A-E).
- From the standard curve, estimate the concentration of the amino acids present in your unknown sample.

 **Results:**

Test tube	Amino acid concentration [ $\mu\text{g/ml}$ ]	Absorbance at 570 nm
Blank		
A		
B		
C		
D		
E		
Unknown sample	_____	

 **Supporting materials:**

- A video explains the mechanism of reaction between ninhydrin and amino acid:  
<https://www.youtube.com/watch?v=P-iK4QUU9t0>
- A video shows the practical steps of the experiment step by step:  
-Part 1: <https://www.youtube.com/watch?v=wvVYF8T7uiE>  
-Part 2: <https://www.youtube.com/watch?v=B4EgYeFqV5Q>